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ABSTRACT

This paper describes open-ended experimentation with the fungus Pilobolus for laboratory work by high school students. The fungus structure and reproduction is described and sources of the fungus are suggested. Four areas for investigation are suggested: the effect of a diffuse light source, the effect of a point light source, the effect of light intensity, and the effect of different colors of light. Questions are also raised that additional experiments could be designed to explore. Also inlcuded is a list of publications concerning additional information on Pilobolus. (BR)

OPEN-ENDED EXPERIMENTATION WITH THE FUNGUS PILOBOLUS

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by

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OPEN-ENDED EXPERIMENTATION WITH THE FUNGUS PILOBOLUS

The study of fungi in high school biology classes is often a rather unexciting phase of the course for most students (and teachers). Classroom and laboratory work with fungi, when present, is usually limited to a study of the comparative morphology of the Ascomycetes and Basidiomycetes, "The Higher Fungi."

Laboratory or field experiments using fungi are even less frequent in high school biology courses. Granted, there are a few standard laboratory activities using fungi, such as mating the plus and minus strains of the common bread mold, Rhizopus, to observe zygote formation. However, from the standpoint of exciting student interest, teachers often find that the standard "canned" laboratory activities involving fungi often fall short of their mark.

There are, however, some relatively unknown fungi that display rather interesting features and that lend themselves quite well to some open-ended experimentation by high school students. One example is Pilobolus and its "Fungus Shotgun."

Pilobolus is a member of the same class of fungi as Phizopus, the Zygomycetes. Like many other members of its class, Pilobolus is a saprobe and a common inhabitant of horse and cow dung. Of particular interest is the mechanism of spore dispersal developed by Pilobolus, in which the entire sporangium (spore case) is violently shot off the sporangiophore and adheres to the first solid object it strikes. The sporangiophores are positively phototropic and eject their sporangia in the direction of light.

Structure and Asexual Reproduction of Pilobolus

The fruit body (asexual reproductive structure) of Pilobolus consists of two main parts, the sporangium and the sporangiophore. The terminally located, black, sporangium contains numerous spores which under suitable conditions may germinate (sprout) to give rise to new plants. The highly specilaized sporangiophore which supports the sporangium consists of the enlarged sub-sporangial swelling and the stalk or stipe. Usually, droplets of "water" are visible over both the subsporangial swelling and the stipe. During development, the subsporangial swelling, acting as a primitive lens, and the stipe function together in the orientation of the sporangium toward light. This is brought about by a growth response which is triggered whenever light entering the subsporangial swelling is brought to focus at any point other than the orange-pigmented area at the top of the stipe. Therefore, through light triggered directional growth of the stipe the sporangium is very accurately aimed at the light source. On reaching maturity, the sporangium is violently discharged ("shot") from the sporangiophore. This is accomplished by means of a "water-squirt" mechanism which is capable of projecting the sporangium to a distance of up to six feet. On discharge, the sporangiophore is thrust against the substrate where, being no longer functional, it disintegrates. However, the sporangium, by means of a ring of mucilage at its base, will stick to any object which it strikes. In nature this will frequently be a nearby blade of grass. Being so deposited, the sporangia are in a good position for being eaten, along with the grass, by a passing horse, cow, etc. The sporangia and spores pass unharmed through

the digestive system of the animal and are excreted. In the fresh, moist dung, the spores within the sporangia germinate to give rise to rapidly growing, vegetative filaments called hyphae. The hyphae grow throughout the dung. After two or three days growth, asexual reproduction is initiated when enlarged, orange-colored, structures, trophocysts, are formed along the hyphae. It is from the trophocysts that the sporangiophore and sporangium develop. This developmental process requires about 12 hours for completion and results in synchronized maturation of the sporangia at approximately noon of the day of discharge. Following the first "crop" of sporangia, new but smaller numbers will develop daily for 2 to 3 days.

Experimenting With Pilobolus

Pilobolus can be easily obtained for observation and experimentation in the classroom or laboratory. For this, simply collect fresh horse (or cow) dung and keep it under moist conditions. After 3 to 4 days, the clear mycelia, sporangophores, and black sporangia should have developed and be plainly visible. A good hand magnifying glass or dissecting microscope will reveal most of the external anatomy of Pilobolus. After examining the gross features of Pilobolus, a number of interesting experiments may be designed around this remarkable organism. The following are suggested areas for investigation and should serve only as guides for the development of other types of experiments:

1. Effect of a diffuse light source. In a room with diffuse lighting, the sporangia will be dishcarged in a random fashion and will adhere to the first object they intercept. This can be observed by

simply covering a transparent collecting dish with another similar dish or a clear food wrap.

2. Effect of a point light source. To test the effect of a single point light source on the phototrophic response of Pilobolus, cover a collecting dish with paper and wrap it in aluminum foil. Make a single, centrally located opening about 4 mm in diameter in the cover. Place clear tape over the opening in order to catch any sporangia that make a "bullseye." After a sufficient number of days has ellapsed to allow for sporangial discharge, remove the covering to observancy pattern. Under these conditions, the sporangia will discharge very directly at the light source, indicating the high accuracy of the phototrophic response mechanism. If concentric lines are drawn at regular intervals around the hole, students could make some mathematical estimates of the accuracy of sporangial discharge.

3. Effect of light intensity. For this experiment, wrap a collecting dish in aluminum foil and then cover it with a semi-opaque paper and make two holes of different sizes. Place clear tape over the holes--again to catch any sporangia that make a "bullseye." Usually, the brighter (larger) light source will be more effective in bringing about a phototrophic response. The growth response of the fruit bodies may also be observed.

4. Effect of different colors of light. To test the effect of light color on sporangial discharge, prepare a collecting dish as in experiment 2 except place two widely separated holes in the covering of the same size and cover with a cellophane paper of two different colors. In this way, you can obtain a rough measure of the effects of

two different wave lengths of light on the phototrophic response. With the use of a greater variety of colors one might establish an 'action spectrum" for Pilobolus.

CONCLUSION

The basically simple experiments described in this paper should merely serve as a starting point for experimentation with Pilobolus. There are numerous other facets of its growth and development which lend themselves to classroom experimentation. Examples of the types of questions around which additional experiments may be designed are as follow: What is the greatest vertical/horizontal distance that sporangia can be shot? What will the sporangia do in the absence of light? Does gravity have any effect on the growth of Pilobolus? What response will occur when two light sources of equal intensity but from opposite directions are focused on a single fruit body? What is the approximate weight of each sporangium and how much force is required to achieve "lift-off"? Statistically, how accurate are the sporangia in reaching their target?

Individuals interested in pursuing these and other questions regarding Pilobolus are referred to the following publications for additional information:

Bland, Charles E. and Thomas M. Charles. 1972. Fine Structure of Pilobolus: Surface and Wall Structure. Mycologia 64: 774-785.

Buller, G. H. R. 1934. Researches on Fungi. Vol. 6, p. 1-189. Longmans, Green, and Co., London.

Page, R. M. 1962. Light and the Asexual Reproduction of Pilobolus. Science 138: 1238-1245.

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